

DETERMINATION OF THE CAUSE OF
MUCOID ENTERITIS DISEASE
IN DOMESTIC RABBITS

by

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INTRODUCTION

Mucoid enteritis is a disease that probably kills more domestic rabbits than any other rabbit disease, accounting for approximately 50 per cent of the mortality during the suckling period. A few years ago, domestic rabbits were not raised in sufficient numbers to be of commercial value; losses due to this disease in rabbits, therefore did not attract attention.

Since attempts have been made to produce rabbit meat in greater volume, the rabbit breeders have been confronted with the problem of losing many rabbits, mostly young ones, due to this disease. This disease may become epizootic and is so classed, because of its peculiar nature, symptoms and post mortem lesions.

Rabbit production is becoming more popular on a commercial basis, because of public demand and it has become a good source of supply of tender tasty meat. People have developed special likings and are showing greater interest in the rabbit, because of its flavor, good taste, tenderness and as a change from the more common meats such as beef, pork or fowl. Thus rabbit production is fast increasing in some parts of the country, and the rabbit breeders are increasing their production by careful selection feeding, and scientific weaning of young litters. Rabbit raising is not only a hobby, but is gaining importance on commercial basis. Many breeders have found rabbit raising a profitable enterprise.

Mucoid enteritis is a rabbit disease which occurs sporadically in rabbit colonies. It usually affects litters between the age of three to eight weeks. Cases are sometimes found in older animals even up to 7 years of age.

The disease synonymously is known as "bloat", "scours", or "diarrhea",

because it produces the symptoms suggested by these names. The abdomen in some of the affected rabbits may be bloated and distended with gas, some times with excessive fluid. Mucous voided with diarrhea is the main feature of the disease, thus the name "mucoid enteritis".

The disease has been considered as the primary cause of heavy financial loss to the rabbit breeder. The disease disappears as mysteriously as it appears. Thus it is difficult to estimate the exact loss and cost on extra nutritive feeding of recovered and weak rabbits.

Previous research gave no encouraging results, because of the difficulty in transmitting the disease experimentally, leaving the workers to guess the cause of the disease.

Present work and studies deal with the attempts to determine the cause of the disease.

REVIEW OF LITERATURE

History of the disease goes as far back as 30 years, when it was discovered in some of the rabbitries in California. The disease was not serious, but was a cause of alarm and attracted the attention of many workers and rabbit breeders. Some time later the U. S. Rabbit Experimental Station at Fontana, California, worked on various experiments, and Templeton (1953), described the disease, giving in tabular form, the results of effect of feeding Aureomycin B-12 supplement on the development of young and young mortality.

While describing the disease he has estimated the loss due to this disease, in excess of 900,000 dollars in the Los Angeles area alone; with this in view, the tremendous financial loss to the rabbit breeders, and to the nation as a whole can be estimated.

Templeton (1953), gave the symptoms as inactivity, lack of appetite, eyes squinted, dull and lusterless, the fur loses sheen and has a rough appearance, the ears are prone, and in the case of albino, the pinkish color is lost, the temperature goes down below normal ($102.7^{\circ}\pm .5^{\circ}\text{F}$). Suffering animals grit their teeth, show intense thirst, and sit close to the water crook, often with the front feet in water, and drink small quantities of water at frequent intervals. Some may be bloated, but the appearance and extent of this symptom are quite variable. The animal may be constipated or have a profuse diarrhea, sometimes voiding a considerable quantity of clear viscid and mucoid material. The face has a pinched appearance, the body becomes shrunken and the young rabbit may lose 20 to 25 per cent of its live weight in 24 to 48 hours.

He describes the post mortem lesions as stomach and upper portion of intestines filled with liquid, occasionally distended with gas or partially digested food. In some cases the lining of small intestines and cecum, shows congestion or redness. Part of the colon or lower bowel frequently contains a large quantity of clear, viscid, mucoid material, while the posterior end of the bowel is usually empty.

In older animals, the disease may be present for a sufficient length of time to produce gross changes. In some of these, the lining of the upper portion of the small intestine may be cheesy, and have ulcerated areas. In other cases lesions are not such, that a distinct differentiation between this malady, and certain types of dysentery can be made. No changes in the lungs, liver or spleen have been found except as a secondary result.

Some of the symptoms described above frequently are mistaken for enteritis, produced by the intestinal type of coccidiosis. There are three types of enteritis most commonly found in young rabbits. Diarrhea present in

ordinary form is known as enteritis, diarrhea stained with blood is known as hemorrhagic enteritis, and the third type mucoid enteritis is seen with symptoms of excretion of clear jelly like substance, gritting the teeth, and presence in the digestive tract of an excessive amount of ingested watery substance.

All previous attempts to reproduce the disease experimentally have failed and the observations indicate, that it is neither infectious nor contagious, and that sanitary measures although always desirable, have little if any effect in preventing its occurrence.

Earlier literature contained in a hand book on Rabbit Raising, put out by the California Agricultural Extension Service, University of California, published by H. M. Butterfield (1950), attributes the cause of the disease, to be a digestive disorder, sometimes confused with coccidiosis, because of the presence of diarrhea. The disease affects all ages, but most often those animals under 18 months. Five to eight weeks old rabbits may die within 24 to 72 hours, but older rabbits carry the disease, for a longer time with less mortality.

Lund (1947), in a publication "Common Diseases of Domestic Rabbits", also gives the cause of the disease as unknown, with the same typical symptoms as described by Templeton.

Lund further states, that few cases are noted in nest box babies, and the greatest incidence (by mortality records), is near the close of the sixth week. Developing and mature stock may also be affected.

Kerriein (1956), in a booklet "Rabbit Nutrition Yesterday and Today", believes that coccidiosis whether described as diarrhea, bloat or intestinal inflammation (enteritis) is still considered the greatest direct cause of domestic rabbit mortality. According to him, the above information is based

on his practical experience as well as observations resulting from controlled studies.

He states that results from early research studies indicated that an alkaline condition in the blood stream was created by high intake of green or root feed. This together with high intake of water proved to favor reproduction (sporulation) of coccidial oocysts.

He said that in some quarters, coccidiosis is recognized, reported, identified and described as either bloat, scours, diarrhea or mucoid enteritis. He has suggested that the rabbit breeders pay more attention to the use of better feed for rabbits, to minimize its spread by creating greater physical resistance by adequate and proper nutrition.

Vail and McKanny (1943), formerly of wild life research, U. S. D. I., described mucoid enteritis or bloat, as a disease quite different from coccidiosis; in the former the cause of the disease is stated to be unknown, whereas the latter has been placed under the parasitic infection caused by coccidia.

Writing about the lesions of coccidiosis infection, they revealed the involvement of liver which becomes hard to the touch and loses its color.

Bargen (1924), in Rochester, Minnesota, had worked on experimental studies on the etiology of chronic ulcerative colitis in humans. He injected gram positive diplococcus organisms intravenously, which he had isolated from the humans suffering from chronic ulcerative colitis, into healthy rabbits weighing about four pounds free from diarrhea. The rabbits after twenty four hours to several days after inoculation developed a violent diarrhea often with blood and mucous in the stools. He likewise isolated along with the diplococcus, a gram negative bacillus which on inoculation in rabbits failed to produce lesions in bowels.

He finally concluded that occasionally localization in the large bowels

of human patients occurs at a certain grade of virulence, when diplococcus strains are passed successively through animals. Green-producing streptococcus isolated from patients with intestinal influenza are found to have a marked affinity for the intestinal tract.

MATERIALS AND METHODS

The diseased rabbits that were used as a source of material for investigation and experiments to determine the cause of the disease were obtained from various rabbitries in the State. A total of eight diseased rabbits (three dead and five alive), were observed and studied for clinical symptoms as a source of material for transmission and then necropsied and observed for pathological changes and lesions. Tissues were saved from all the vital organs for histo-pathological studies.

Bacteriological Studies

The bacterial flora of the intestinal tract of normal rabbits was studied at the same time, in order that a comparison to that of the diseased rabbits could be made.

Each sample of feces from eight diseased rabbits and five normal rabbits was emulsified with normal saline solution in a sterile mortar and pestle. Loopfuls of the fecal suspension thus obtained were inoculated on blood agar and tryptose agar plates. Thioglycollate media and nutrient broth were also inoculated. All the inoculated media was incubated at 37°C for 24 hours. Blood cultures were also made from the blood of diseased rabbits on tryptose agar and blood agar plates.

Smears were made and stained by Grams method from the growth obtained

in the tubes or on the plates, and examined under the microscope for staining and morphological characteristics of the organisms.

Biochemical Studies in Media

Biochemical studies were made by inoculating different sugars viz: dextrose, maltose, mannitol, lactose, sucrose and arabinose with the organisms isolated from each of the eight diseased rabbits and five normal rabbits. Other media inoculated were nitrate agar slants on surface, gelatine stab, motility tube with straight needle, litmus milk, lead acetate and three M.R.V.P. tubes (Methyl Red Voges Proskauer), with a control in each case.

The sugar tubes were observed after 24, 48, 72, 96 hours and seven days incubation for acid or acid and gas formation.

Motility was recorded after 24 hours incubation. A hanging drop method was adopted in determining the motility by taking a loopful of growth from the cultured motility tube and mixing with a drop of saline on cover slip. This was fixed on a hanging drop slide by vaseline on sides of cover slip. The slide so fixed was examined under the microscope with low and high power.

Gelatine liquefaction was determined after 24 hours incubation by keeping both inoculated and control tubes in the refrigerator for five minutes, and then leaving in a room temperature until the time when slight melting occurred. The inoculated tube was compared with the control tube for intensity of liquefaction of gelatine by organisms, and rated as to whether partial, complete, or no liquefaction.

Litmus milk tubes were observed after 24 to 48 hours incubation for reduction of the litmus.

Nitrate agar slants inoculated on surface, were tested for the property of reducing nitrates into nitrites after 24 to 48 hours incubation. The

test was done by adding equal quantities of dimethyl alphanaphthyl amine solution and sulphanilic acid solution in the culture and control slants, and comparing the dark pink color formation in the two tubes. The formation of pinkish color after mixing the two chemicals in a culture growth would indicate the property of organisms to reduce nitrates into nitrites. There will be no change in the control tube.

Methyl Red Voges Proskauer media were tested after 24 hours incubation for indole, Voges Proskauer and methyl red.

The indole test was made by taking 5 ml of culture and adding to it 1 ml of ether to form a ring on the upper layer of the culture. Kovacs reagent (prepared by mixing 5 gr p-dimethylamino benzaldehyde, 75 ml amyl alcohol and 25 ml concentrated hydrochloric acid), 0.2 to 0.3 ml was added slowly on the sides of the tube so as to form a middle layer in between the culture and the ether. A pinkish brown ring formation between the two layers would indicate the property of indole forming bacteria. No reaction would indicate negative for indole.

Methyl red test was made by adding 5 ml of culture media to 5 drops of methyl red solution (prepared by mixing 0.1 gm bacto methyl red in 300 ml of 95 per cent alcohol and further diluting to 500 ml with distilled water). A red pink color formation after mixing culture media and methyl red would indicate no color formation. The negative mixture would turn into deep yellow color.

Voges Proskauer test was done by mixing in 1 ml of 24 hours culture media, 0.6 ml of V. P. reagent (prepared as 5 per cent α -naphthol in absolute alcohol), and 0.2 ml of 40 per cent potassium hydroxide. A brownish red color ring formation would indicate organisms positive for V. P. test, no change would indicate negative test.

Lead acetate tubes were observed after 24 hours to seven days incubation for formation of brown or black color in slants. This indicated the ability of organisms for liberating hydrogen sulphide. No color formation would indicate the test as negative.

Transmission Studies

Healthy rabbits irrespective of breed, sex, age or weight were used in the experiment for transmission studies. In each experiment of transmission, the rabbits were divided into four groups as shown in Table 1. Saline washings were prepared from the intestinal tract of diseased rabbits by scraping the mucosal surface of the intestines. This washing was administered to one group of 13 rabbits by means of a stomach tube into the stomach. Bacterial suspensions were prepared from tryptose blood agar plates that had been inoculated from the intestines of diseased rabbits, and were also administered by means of a stomach tube into another group of five rabbits.

Table 1. Grouping of rabbits under experiment of transmission.

No. of healthy rabbits administered	No. of healthy rabbits administered	No. of healthy rabbits injected	No. of healthy rabbits injected
Diseased: Rabbit No.	sterile fecal material through stomach tube	sterile bacterial suspension through stomach tube	with blood serum; with bacterial suspension I/P
Group I	Group II	Group III	Group IV
11936	5 A, B, C	1 A	-
12008	3 D, E, F	1 B	-
12101	2 G, H	1 C	1 A
12190	1 I	1 D	1 B
12903	1 J	1 E	1 C
12904	1 K	-	1 D
12905	1 L	-	-
12906	1 M	-	-
Total of healthy rabbits used	13	5	4

Blood from rabbits ill with mucoid enteritis was injected intraperitoneally into a third group of four rabbits. A fourth group of four rabbits was inoculated intraperitoneally with a saline suspension of bacteria.

Three dosages of stomach and intestinal washings were administered through a stomach tube to each rabbit under group I and II. Three injections of bacterial suspensions were also given to each rabbit through the stomach tube under group IV, and only one injection of serum was given intraperitoneally in each rabbit under group III. Two rabbits, viz. J and K in group I, were administered with intestinal suspension of diseased rabbits Nos. 12903 and 12904 directly into the stomach through surgical operation laparotomy.

Four of the rabbits on experimentation in Table 1 under group I showed ill effects and were studied for clinical symptoms and post mortem changes. These were further used for transmission experiments on some other healthy rabbits as shown in Table 2 below.

Table 2. Group of rabbits used for second transmission experiments.

Rabbit No. under experiment in group I, Table 1	No. of new healthy rabbits : injected I/V with blood : serum obtained at the : height of temperature of : 105° F	No. of new healthy rabbits : administered with fecal : material through the : stomach tube
1	2	3
A	1 (a)	-
D	-	1 (a)
F	1 (b)	-
I	1 (c)	1 (b)
Total healthy rabbits used	3	2

Temperatures were recorded 24 hours before and seven days after the administration of the fecal or bacterial suspension, and all the rabbits under experiment were kept under observation for development of any sign or symptoms

for 30 days. These rabbits were also kept under a restricted diet of more lettuce and restricted intake of pellets, during the period of observations.

Studies for Presence of a Virus

A series of six passages of seven and ten days old chick embryo eggs was maintained by inoculating chorioallantoic method with a filtrate to determine the presence of a virus. The filtrate was obtained by first centrifuging the fecal suspension in a centrifuging machine at 3000 rpm for 20 minutes, and then filtering the supernate in Jenkins filter. Two sets of eggs each with 24 eggs were inoculated with varying doses of filtrate, one set with 0.5 ml and the other with 0.2 ml. Twenty-four control eggs were inoculated in the same way, 12 with the unfiltered fecal suspension and 12 with sterile normal saline solution. Thirty six, three weeks old mice were used for attempting to isolate a virus in the filtrate. Twenty four control mice were also used, 12 with unfiltered fecal suspension and 12 with normal saline solution. The dose in mice used was 0.01 ml intracerebrally.

Blood Studies of Normal and Diseased Rabbits

Blood studies were made in normal as well as diseased rabbits under transmission experiments. Red blood corpuscles count, white blood corpuscles count and differential counts were made on these animals. Sodium citrate 0.2 per cent was used to prevent coagulation of the blood. The blood was obtained direct from the heart in a syringe and was then transferred to the test tube containing 0.2 per cent sodium citrate.

Histo-pathological Studies

Rabbits that died or were sacrificed during study were necropsied and

the animals examined for gross pathological lesions. Tissues were saved for microscopic examination by fixing in 10 per cent formaldehyde, dehydrating, sectioning and staining with hematoxylin eosin stain. There were received preserved body tissues of eight cases of mucoid enteritis from Iowa State and these were also studied for histo-pathological changes.

Bone marrow was studied for changes that may have been due to mucoid enteritis. Smears of bone marrow were made on clean glass slide and stained by two different methods, one by Wright's method and the other by Giemsa. Sections of bone were also studied microscopically.

Studies for Presence of Parasites

The fecal samples were examined for the presence of coccidiosis according to the method described and adopted by C. A. Slanetz, Department of Pathology, College of Physicians and Surgeons, Columbia University, under the caption "Rabbit Coccidiosis Method 1954", (method for examination of rabbit for presence of coccidial oocysts).

Survey Studies

A questionnaire was prepared and sent to 80 members of the Kansas State Rabbit Breeders Association in order to collect additional data and information on the incidence, occurrence, frequency, mortality, losses and control of mucoid enteritis in their rabbitries. The form of the questionnaire is given in the Appendix.

RESULTS

The rabbits with mucoid enteritis were observed for clinical symptoms and post mortem changes.

The rabbits most frequently affected were between the age of three to eight weeks. The first symptom frequently was loss of appetite, showing intense thirst, pale in color, dull eyes, not inclined to move, tendency to remain in a corner with arch back, rough hair coat and with prone ears. The above symptoms are well illustrated in pictures vide Plate I, Figs. 1 and 2. One rabbit had its fore feet in the water crock, drinking small quantities of water frequently. In all affected rabbits, there was found a constant flow of mucous material clear and viscid in nature from the anus. The perineal region became matted and soiled with mucous, as could be seen in a picture vide Plate II, Fig. 1. The animal when handled was found to have bloat with excessive fluid and gas, splashing sound could be heard, when the animal was examined close to the ear. Two affected rabbits, Nos. 12101 and 12190, had profuse diarrhea but with little mucous. The temperature recorded in all rabbits was sub-normal 100.5° to 101°F , normal temperature of rabbits being $102.7 \pm .5^{\circ}\text{F}$.

Necropsy findings in all the eight affected rabbits revealed no gross lesions except that the cecum and colon were full of gas, and watery blood stained mucous. Several hemorrhagic spots were observed on the mucosal surface of the large intestine. The small intestine was also full of gas and mucous material mixed with feces. The mucous membrane of small intestine was congested, reddened and oedematous vide Plate II, Fig. 2.

Other internal organs viz. spleen, kidney, liver, gall bladder, pancreas, genital organs, bladder, heart and lungs were found with absence of gross lesions.

The organisms that were isolated from feces of normal as well as diseased rabbits are shown separately in tabular form with biochemical changes in different media.

EXPLANATION OF PLATE I

Fig. 1 and 2. Photographs of rabbits infected with mucoid enteritis, showing typical symptoms of tendency to remain in a corner with arch back, dull eyes, rough hair coat, prone ears.



Fig. 1



Fig. 2

EXPLANATION OF PLATE II

- Fig. 1. Photograph of rabbit infected with mucoid enteritis, observed with the perineal region matted and soiled with mucous.
- Fig. 2. Several hemorrhagic spots observed on the surface of the cecum and colon, which are full of watery blood stained mucous and gas. Small intestines congested and reddened.



Fig. 1



Fig. 2

Table 3. Description of morphology and biochemical reactions of organisms isolated from the feces of normal rabbits.

Rabbit No.	Bacterial Colony Characteristics:	Morphological characteristics:	Dextrose	Mannitol	Lactose	Sucrose	Arabinose	Litmus milk	Motility	Indole Test	M.R. Test	V.P. Test	Nitrite from Nitrate	Gelatin Stab	Lead Acetate	Classification of organisms	
1	Gray oval large raised	Long Gram + rods	-	-	-	-	-	-	+	-	-	-	-	-	-	Bacillus Pasteuri	" indicates not done.
2	White small round raised	Fat Gram + cocci	+	+	+	+	+	+	+	-	-	-	-	-	-	Sarcina ureae	+ indicates positive.
3 (a)	Smooth grayish	Small Gram - rod	-	-	-	-	-	-	-	-	-	-	-	+	-	Unidentified	- indicates negative.
(b)	Raised round grayish	Gram + cocci	-	-	-	-	-	-	+	-	-	-	-	-	-	Sarcina flava	+ indicates negative.
4	Transparent watery golden yellowish	Small Gram + rods	-	-	-	-	-	-	+	-	-	-	-	+	-	Unidentified	- indicates negative.
5 (a)	Round small golden yellowish	Gram + cocci	+	+	+	+	+	+	-	+	+	-	-	-	-	Sarcina maxima	+ indicates negative.
(b)	Dark grayish	Gram + rods round ends	-	-	-	-	-	-	-	-	-	-	+	+	-	Unidentified	+ indicates negative.
(c)	White small round raised	Gram + cocci	-	-	+	+	+	-	-	+	+	-	+	-	-	Staphylococcus albus	- indicates negative.
(d)	Grayish yellow	Gram + cocci	+	+	-	-	-	-	-	-	+	+	-	-	-	Streptococcus faecalis	+ indicates negative.
(e)	Dark tan sticky layer forming	Oval shape Gram + rods	+	-	+	+	+	+	-	+	+	+	+	+	-	Unidentified	+ indicates negative.

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		Dextrose	Mannitol	Lactose	Sucrose	Arabinose	Lithmus milk	Motility	Indole Test	M. R. Test	V. P. Test	Nitrate from Nitrite	Gelatinase Stab	Lead Acetate	Classification of organisms
Rabbit:	Bacterial	:	:	:	:	:	:	:	:	:	:	:	:	:	:
No. :	Colony :	Morphological characteristics:	:	:	:	:	:	:	:	:	:	:	:	:	:
:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
12101*	(a)	Glistening yellow with sweet odor	very minute Gram + reds	⊕	⊕	⊕	+	-	-	+	+	+	+	+	Unidentified
	(b)	Raised dark round with layer formation	small fat Gram + bacilli	⊕	⊕	-	+	-	-	-	+	+	-	-	do..
	(c)	Seamy growth pin pointed transparent colorless green-spherical bodies producing	Gram + cocci in pairs and short chains with	⊕	⊕	"	+	-	-	+	-	-	-	-	Streptococcus bovis
	(d)	Raised round grayish white	very small Gram + reds	⊕	⊕	⊕	+	+	-	+	+	+	+	+	Unidentified
	(e)	Glistening white continuous round raised and granular	Spherical cocci in Gram + in clusters	⊕	⊕	-	+	-	-	+	+	+	-	-	do..
	(f)	Yellow off shooting and branching	Very small cocci like Gram + reds with rounded ends	-	-	-	-	+	-	-	-	+	-	+	do..

* Six organisms isolated
⊕ Acid and gas formation
+ Negatively positive
- negative

Various organisms Nos. 11936, 12008, 12190, 12101 (a) (c) and (d), 12903, 12904 isolated from the corresponding diseased rabbits, when introduced into other healthy rabbits by means of stomach tube and parenteral injection, did not produce any sign of illness.

Results of Transmission Experiment

Group I. Rabbit A an adult, when given a fecal suspension through the stomach tube, was found to have developed no apparent symptoms except that there was a rise in temperature 104°F after second feeding. The thermometer when removed from the rectum was stained with fluid feces. The temperature was 105°F on third feeding and which continued for three days. Other typical symptoms of mucoid enteritis were absent. At the height of temperature, the rabbit was bled from the heart, and the blood injected into another rabbit, No. (a), through the ear vein as shown in Table 2, column 2.

The injected rabbit failed to show any rise in temperature or symptoms or signs of illness.

Rabbit B and C, 16 weeks of age, were found with no changes on introduction of fecal material, except that the temperature recorded for five days varied from 103.2° to 103.8°F . These rabbits were kept under observations for 30 days.

Rabbit D an adult, was given a suspension of fecal material through the stomach tube, and after the second feeding exhibited hard labored breathing, but recovered and lived for 36 hours. During this period, the rabbit showed many of the same symptoms as those observed in mucoid enteritis, dull looking, squinted eyes, fore feet in water, hard breathing, off feed with no change in temperature and absence of mucous or diarrhea. Necropsy revealed bloating of the colon with absence of hemorrhage or mucous on the mucosal surface.

The liquid feces collected from this dead rabbit was suspended in normal saline solution and fed to rabbit No. (a) under column 3 in Table 2. This animal remained normal and showed no evidence of disease.

Rabbit E, 16 weeks of age, on feeding of fecal material through the stomach tube failed to show signs of illness except that the temperature recorded for four days was in variation of 103.4° to 104.2° F. The rabbit was kept under observation for 30 days.

Rabbit F, an adult, on introduction of fecal suspension failed to develop any signs of illness, except a high temperature of 105° F. The rabbit was bled from the heart at this stage, and injected into another rabbit through the ear vein. The injected rabbit No. (b), as is shown in Table 2, column 2 remained normal. The temperature of 105° F, in F rabbit continued for three days, and was alright when observed for 30 days.

Rabbit G 16 weeks of age, on third feeding had developed difficult breathing, with no change in the temperature. The rabbit died after 12 hours and on necropsy, no marked change or gross lesions were found in bowels except that the lungs were congested and consolidated and sank, when a piece was placed in a beaker containing water. This may be due to fecal suspension having gone in lungs and caused consolidation.

Rabbit H an adult, after feeding of fecal suspension was observed for 30 days with no marked changes.

Rabbit I an adult, on introduction of fecal suspension had a temperature of 105° F for three days. It was bled from the heart, and the blood was injected through the ear vein into rabbit No. (c) as shown in Table 2, column 2, without showing any significant clinical changes. The rabbit I subsequently died after fourth feeding. Upon necropsy consolidation of both lungs was observed, the intestines were distended with liquid feces with

no tint of mucous or diarrhea. The feces collected was administered into another rabbit No. (b) four months old as shown in Table 2, column 3, through the stomach tube with no development of any signs of illness.

Rabbits J, K, L and M, all 16 weeks of age, were observed for 30 days and did not show any sign of illness. Rabbits J and K were inoculated direct into the stomach with the fecal suspension by means of a laparotomy, opening the abdominal cavity and injecting the material into the stomach. In other two L and M the fecal suspension was administered through the stomach tube. There were observed no symptoms or change in temperature.

Group II. Rabbit A an adult, when administered with the bacterial suspension, did not develop any symptoms and appeared normal during the observation of 30 days.

Rabbit B an adult, on introduction of bacterial suspension had a temperature of 104°F after second feeding, was slightly dull with loss of appetite, but there was no presence of diarrhea. The animal subsequently recovered, started eating well with normal temperature. The rabbit was kept under observation for 30 days.

Rabbits C, D and E all adults, when introduced with the fecal suspension did not show any ill effects during the period of 30 days observation.

Group III and IV. Rabbits under group III inoculated with the blood serum I/P and group IV inoculated with bacterial suspension I/P, were observed for 30 days with no change.

Results of Virus Studies

A filtrate as described under materials and methods, failed to kill 7 and 10 days old 48 chick embryos. Chorioallantoic fluid was harvested and

inoculated into another group of chick embryos using 24 eggs every time for six passages, with no end point of LD₅₀. Thus no desired results were obtained even after six passages. Twelve control chick embryos in each case, which were inoculated with unfiltered fecal suspension were found dead within 12 hours, where as another set of 12 control chick embryos which were inoculated with sterile normal saline solution were found without deaths.

Negative results were also obtained, when 36 mice were injected with the filtrate intracerebrally. Twelve control mice which were inoculated with the unfiltered fecal suspension were found unthrifty with no deaths, whereas 12 control mice which were inoculated with normal saline solution were found without any effect.

Results of Blood Studies

There was no apparent change in the blood picture, when examined for various values. The white blood cells count in one of the diseased rabbit No. 12904 was 3650 per ml as compared with 5000 to 9000 per ml in other diseased rabbits. In normal rabbits they varied from 7950 to 9650 per ml. There was found a slight hemo-concentration in two rabbits Nos. 12903 and 12904. The red blood cells count in these rabbits varied from 5,400,000 to 6,500,000 per ml as compared with 5,070,000 to 5,880,000 red blood cells count per ml in normal rabbits. There was no change in the form, shape or size of red blood cells.

Histo-pathological Results

The study of bone marrow of diseased rabbits, revealed no change, when compared with the bone marrow of normal rabbits. In the bone marrow slides of rabbits Nos. 11936 and 12008, the cells and the connective tissue appeared

cloudy whereas slides of normal and other diseased rabbits had a clear and bright appearance.

Microscopic examination of tissue sections of various organs revealed no significant changes between the normal and diseased rabbits.

Infiltration of leucocytes and edema of mucosal surface of the digestive tract was found as histo-pathological change in the diseased rabbits.

Results of Parasites Studies

Coccidia were not found in the rabbits infected with mucoid enteritis. Fecal suspension and tissue sections were used for this study.

Survey Results

Forty-one replies to questionnaire that were received from the various members of the Kansas State Rabbit Breeders Association were of considerable value as to information on the disease. The information compiled is as follows:

Incidence. The disease is prevalent in every part of the State, as well as adjoining states viz. Iowa, Missouri. It is more or less wide spread throughout the country.

Susceptibility. All breeds of rabbits were found susceptible. The breeds that are raised by the breeders are shown below, and the age at which young females are bred.

New Zealand White 6 months, Sandy Flemish Giant 8-9 months, California 6-7 months, Blue Dutch 4-5 months, Polish 5-6 months, New Zealand Reds 6 months, American Chinchilla 6-7 months, Satins 4-5 months, American Dutch 6-7 months, Havana Dutch 6 months, Dutch Blue Black Chochlate 10-12 months,

New Zealand White Giant Chin 6-8 months, Checkers 5-6 months, Silvers 6-7 months, Champagnes 6 months.

However one breeder reported that certain strains of rabbits seem more susceptible. He had some inbred Chocolate Dutch which were very susceptible, and some inbred Blue England Spots which seemed to be comparatively more susceptible.

Occurrence. The disease may occur at all times of the year, but late fall and early spring have been found more favorable for appearance of the disease. Quite a few cases have been reported in summer when it is extremely hot.

Frequency. The disease has been frequently reported in some rabbitries, while some growers have never encountered the disease. Breeders report that the disease appears once or twice, and each time one or more litters are affected. One rabbit may be found dead in the morning, the other one or two may be found in the evening. It may occur after a short time after the previous outbreak or may not be encountered again.

Feed. Various commercial feeds are being used by the rabbit breeders.

Mortality and Losses. Some breeders reported that the older animals would recover in 3-4 days. The disease is fatal to 3-6 weeks old rabbits. The mortality in young rabbits is relatively higher than in the older animals. It may be 50 to 100 per cent in rabbits of 3-6 weeks age, but fewer losses have been recorded in older rabbits also, the mortality rate being 1 to 5 per cent. Animals that have recovered may again succumb to the disease. Their growth is stunted by the disease condition. One breeder during his rabbit raising since 1922, lost 800 out of 1000 litters affected. The older

rabbits take about 3-10 days to gain full fryer weight, whereas young litters require extra feeding for at least 2-3 months.

Course. The course of the disease has been reported to be very short, young litters die within 3-12 hours after symptoms are observed. The disease may appear mysteriously, and disappears the same way.

Incubation Period. As the disease is not transmissible under experimental conditions, the incubation period is not definitely known.

Symptoms. The symptoms are reported practically the same as recorded and described above. In addition young litters have more tendency to drink water at frequent intervals, rabbits sit in hunched position in a corner, fur looks coarse, would die within 3-12 hours.

Control and Treatment. Some breeders have reported aureomycin with some success in older rabbits, 100 mg at 24 hours interval for three days have given good results. This is of little value in young litters, due to the fact that they die within a matter of few hours. Some breeders say, they obtained a red medicine from some Veterinarians (name of the medicine they don't know) and had obtained good results, on feeding of the drug. Others have tried a tree limb bark about 3 feet long, cut in half, kept along with feed. Others have tried a small amount of Liquid Bluing mixed in small amount of water twice a day, for few days with feeding of little hay only, pellets started after few days. Many give dry milk or yeast in daily feeding at time of kindeling to eight weeks, and they don't give greens. Some feed prairie hay, others feed crackers.

Precautionary measures seem to be of little value in most of the outbreaks. However some tried removing the pellets and the oats, water crocks

and salt spoils. The premises and the housing of the rabbits have been kept clean with no presence of any dampness or draftiness. Many others believe, the best method of control is, to do away with infected rabbits. Disinfection of the premises has always been tried by the breeders but apparently is of little value. Some isolate all sick rabbits, remove them to another place away from the rabbitry. Breeders have tried to keep their rabbitries free from flies and kept their hutches, feeding and drinking jars clean, but still have encountered mucoid enteritis.

DISCUSSION

Five of the rabbits under transmission experiments manifested some of the symptoms, such as rough coat, dullness, inappetance, feet in watering vessel, that are also observed in mucoid enteritis. But it may be discussed that the symptoms were not of mucoid enteritis because of absence of other typical symptoms and high temperature. In actual infection we find the temperature goes down below normal. The high temperature is probably due to other factors possibly secondary infection, or possibly due to suspension having gone in lungs and caused pneumonia with rise in temperature. The rabbit was bled at the height of the temperature and the blood injected into another rabbit did not produce any symptoms of disease. There was no mucus or diarrhea present. Because of difficulty in transmitting the disease experimentally, it may be said that the disease is of a non-contagious nature. Isolation of different types of organisms from the diseased rabbits and introducing such pure cultures directly into the digestive tract, did not give satisfactory results. The organisms thus isolated may be assumed to be non-pathogenic and not directly associated with the disease.

The feces collected from the bowels of rabbits under experiment, which showed some symptoms when introduced into another group of healthy rabbits failed to reproduce these symptoms. Blood serum obtained from the diseased rabbits Nos. 12101, 12190, 12903, 12904, also did not show any development of symptoms, on inoculation into healthy rabbits.

In an attempt to transmit the disease 31 healthy rabbits were used for transmission studies. These studies indicate that the disease cannot be readily reproduced by using a filtrate and various bacteria isolated from eight fecal cases of mucoid enteritis.

The previous attempts by other workers have also failed to reproduce the disease. Since the disease was not reproduced in these studies, no definite statement can be made as to the etiology of the disease.

These experiments were so planned to include as many routes of transmission as possible viz. through stomach tube, through parenteral administration, through surgical operation to ensure that the fecal material introduced was directly into digestive tract, but the disease entirely could not be produced. The reasons for these failures may be attributed to the bacteria present losing their virulence, when handled artificially outside the body or symbiosis may be necessary or other unknown factors.

One species of *Streptococcus* (*Streptococcus bovis*), was isolated from five out of eight cases of mucoid enteritis. This organism was not isolated from normal rabbits. There is a possibility that this organism may be associated with this disease entity, since other workers were able to reproduce an enteritis in rabbits with the streptococci isolated from human cases of chronic ulcerative colitis.

The streptococci when grown on culture media using tryptose agar and

blood agar were first observed with scanty growth, pin-pointed, transparent, colorless colonies with production of green-producing of mucous nature after 48 hours incubation. The organisms were non-hemolytic changing from greening (alpha) to no observable change (gamma). The organisms have no property of producing indole or reducing nitrates into nitrites. They produce acid from dextrose, maltose, mannitol, lactose and sucrose, litmus milk turned acid, curdled in 3-5 days, and there was no liquefaction of gelatine. The organisms are non-motile, stain gram positive, appear in pairs or short chains with spherical shape. The organisms were chemical tolerant to a 2 per cent salt concentration.

The pathological changes observed in the sections of the intestine indicated that considerable damage occurs to the mucosa and sub-mucosa. This damage may be due to a toxin produced by bacteria or within the lumen of intestine. No change was observed in other body tissues studied for histopathology. Slight cloudy appearance in bone section could be due to no good staining or keeping the tissue for long time in the refrigerator. Hemocentration in blood study of the two diseased rabbits could be due to loss of fluid content of blood in diarrhea. Low count of white blood cells in one rabbit could be due to an individual having less leucocytes.

It was not possible to duplicate the work of Bargen who was able to reproduce diarrhea in rabbits with streptococci isolated from the intestinal scrapings of humans suffering from chronic ulcerative colitis.

The thought of the possibility of coccidiosis infection was not seriously considered as one notices bloody or blood stained diarrhea, besides one finds the presence of oocysts in fresh feces. Absence of coccidia in feces and intestinal tissue sections, and also absence of typical lesions in the liver of the diseased rabbits under study were quite suggestive that mucoid

enteritis and coccidiosis are two different separate entities.

The possibility of mucoid enteritis being caused by bacteria or a virus is questionable, as an introduction into the body should cause the disease, unless the ideal environments are not present in the digestive tract, or the viruses are inactivated due to unknown reasons. Failures to isolate a virus on chorio allantoic membrane of chick embryos and in cerebral tissue of mice are suggestive that a virus has little role in producing the condition mucoid enteritis. It may be possible that virus may facilitate the action of the organisms, or create conditions in body for lower resistance thus paving the way for organisms to produce its effect.

Taking in view of Bergen's results in producing diarrhea in rabbits following injection of diplococci (streptococci), which were isolated from the human feces suffering from chronic ulcerative colitis, gives an indication of the possibility of streptococcus being associated with mucoid enteritis.

From the survey made from the rabbit breeders, and the diseased rabbits that were used as a source of investigation, it was apparent that mostly young litters were the victims of mucoid enteritis. When the disease appears, one or more litters are affected. Sometimes only one litter, sometimes an older rabbit may be found to have mucoid enteritis.

One cannot think of possibility of mothers milk being toxic to litters, because the disease has been observed not only in litters but in older animals, in one case as old as 7 years reported by Templeton (California), 1953.

There is apparently no direct relationship between feeds or protein content as indicated by the survey.

Breeding does at an early age has no correlation with incidence of mucoid enteritis. Horreleins statement that using does for breeding before their puberty period would result in production of litters with impaired resistance

seems to be without foundation. Breeders have been using does as early as 4-5 months, although the breeding period depends upon the breed of rabbit, but no doe can be bred earlier than sexually mature.

Climatic conditions have been mentioned by many breeders as a factor, although the disease may occur at any time of the year.

SUMMARY

Transmission studies were made to determine the cause of mucoid enteritis.

A total of eight diseased rabbits was examined and studied for clinical symptoms, which were loss of appetite, rough hair coat, dull eyes and prone ears. Post mortem lesions were only found in digestive tract which was full of mucous and gas, looked hemorrhagic. Mucous membrane of the intestines was found congested and reddened. Other organs revealed no gross changes.

Histo-pathological studies revealed no apparent change in many vital organs viz. liver, lungs, heart, spleen and kidney, except in the digestive tract which was found to have edema of sub-mucosa, and infiltration of leucocytes and sloughing of mucosal cells. Blood changes were not marked except that hemoconcentration in two diseased rabbits was found due to losing fluid content of blood in diarrhea. Bone marrow slides and bone sections did not show any change.

Various organisms were isolated from feces of normal as well as diseased rabbits. The morphological, physiological and immunological characteristics of these organisms were studied.

The isolation of the etiological agents was also attempted by inoculation of 7 and 10 days old chick embryo eggs and three weeks old mice, but no virus could be detected.

Studies made indicate that the coccidia are not the etiological agent in

mucoid enteritis.

The disease entity could not be reproduced by various transmission experiments viz. by introducing the fecal suspension directly into the stomach through a stomach tube and laparotomy, and by injecting the bacterial suspension intra-peritoneally and through stomach tube. In the 31 rabbits that were used, the disease could not be reproduced.

Clinical symptoms of mucoid enteritis were not observed in rabbits, that were used for transmission experiments, except that there was a rise in temperature, with some of the symptoms, but further transmission studies could not reproduce the symptoms.

Survey studies were of considerable value as an additional data and information as the incidence, occurrence, involvement of different breeds with breeding age of young females, frequency, mortality and losses, incubation period, course, symptoms and control and treatment aspects of the disease are concerned. As regards the cause of the disease, many breeders were anxious to know the cause. They however believed from their experience, that climate is also one of the factors that could be responsible for the appearance of the disease; extreme hot or extreme cold with dampness favored the incidence and occurrence of the disease.

The disease is non-contagious in nature, occurring in young litters of three to eight weeks of age affecting one or two rabbits, has a very short acute course, causing losses within few hours of appearance of the disease.

Streptococcus bovis organism was isolated from five out of eight diseased rabbits. This organism did not produce any symptoms when introduced in some of the healthy rabbits. But considering the results obtained by Bargen in producing diarrhea in rabbits following injection of diplococci (streptococcus), which was isolated from the human feces suffering from

chronic ulcerative colitis, it gives an indication of the possibility of streptococcus group being associated with the condition in mucoid enteritis.

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APPENDIX

(The information which you provide will be kept confidential)

Data and Information
on
Mucoid Enteritis in Rabbits

1. Do you maintain a rabbitry? Yes () No ()
2. How long have you been associated with the rabbit industry? _____
3. Is rabbit breeding your profession () or hobby ()?
4. How many rabbits do you maintain in your rabbitry? _____
5. What breed or breeds do you maintain in your rabbitry?

6. At what age do you breed the young females? _____
7. Please state the number of rabbits of different ages in your rabbitry:
3-8 weeks _____ 8-16 weeks _____ 16 and over _____
8. In what age groups above have you found mucoid enteritis in your rabbits?
3-8 weeks () 8-16 weeks () 16 and over ()
9. What symptoms have you observed? Please check:

Thirst	()	Not inclined to move	()
Loss of appetite	()	Urination	()
Paleness	()	Droopy	()
Diarrhea - watery	()	Place fore feet in	()
Diarrhea - mucoid	()	water vessel	()
10. What percentage of rabbits which were once infected have recovered?

11. What percent loss do you estimate due to mucoid enteritis in rabbits (deaths)? Please check one:

1-5%	()	20-30%	()
5-10%	()	30-50%	()
10-20%	()	50-100%	()
12. After symptoms of mucoid enteritis have been noticed, how long afterwards has it taken sick animals to recover? _____
13. What ration or brand of feed do you use? _____
14. How many rabbits have been infected with mucoid enteritis since you started raising rabbits? _____

15. What is the total death loss of all diseases in your colony?

1-5%	()
5-10%	()
10-20%	()
30-50%	()

16. What disease takes the greatest toll in your rabbitry? _____

17. Is the above disease very common or does it occur only occasionally?

18. How many rabbits do you find infested with this disease each time it occurs? Please check: 1 rabbit (); 1 litter (); two or more litters ().

19. Have you ever consulted any rabbit expert or a veterinarian to treat the sick rabbits or look into the disease? _____

If so, with what results? _____

20. What precautionary measures do you adopt when the disease makes its appearance? _____

21. Do you consider weather a factor on cause or control of the disease?

22. Is the disease fatal to mature rabbits _____

23. Please list any other information that you think may be useful to us in attacking this disease problem.

Place:

Signature:

Date:

Address:

DETERMINATION OF THE CAUSE OF
MUCOID ENTERITIS DISEASE
IN DOMESTIC RABBITS

by

HARIRAM ALIMCHAND SHIVHANI

Graduate of Bihar Veterinary College, Patna, India, 1950

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

Mucoid enteritis has become an economical problem to rabbit producers, since the value of rabbit meat has become so great. As breeders have attempted to meet the increased demand for fryers, they have been faced with the problem of losing as many as 80 per cent of the young during the weaning period. The losses have been primarily attributed to mucoid enteritis which takes heavier toll of the young than any other disease of domestic rabbits. The disease was first reported in California and has been prevalent in the country for several years. The financial loss to the breeder in this country may be estimated by looking at the yearly annual loss in the Los Angeles area alone, which has been estimated at 900,000 dollars. The cause of the disease is still unknown, which makes control difficult. Previous attempts to find the cause of the disease were not successful. This study was therefore undertaken to attempt to determine the cause of the disease.

The diseased rabbits that were used as a source of material for investigations were obtained from various breeders in the state. Clinical symptoms and post mortem lesions were studied in these rabbits. Intestinal saline washings and scrapings from these rabbits were introduced into a group of healthy rabbits via a stomach tube. Another group of rabbits was given the fecal suspension directly into the stomach by laparotomy. Blood collected from diseased rabbits was injected intraperitoneally in a second group of healthy rabbits.

Blood cultures were made from the blood of diseased rabbits in broth, and thioglycollate tubes, and tryptose and blood agar plates. Bacteriological inoculations were also made from the intestinal contents. Bacteriological studies were also made of the feces from normal rabbits. Various bacteria were isolated from these samples, and the organisms isolated from the feces of the diseased rabbits were suspended in normal saline solution and

administered by means of a stomach tube into a third group of healthy rabbits. A fourth group of rabbits was inoculated intraperitoneally with each bacterial suspension.

Studies for the presence of a virus were made by inoculating a filtrate of fecal suspension into seven and ten days old chick embryos by the chorio-allantoic route. Twenty four eggs in each case with a varying dose of 0.5 ml and 0.2 ml of the filtrate were used. A similar number of controls were also employed using unfiltered fecal suspension and sterile normal saline solution. Intracerebrally, inoculations were also made into 36 mice, three weeks of age. Controls were also inoculated with the unfiltered fecal suspension and normal saline solution.

Examinations for the presence of coccidial parasites were also made.

The blood in normal as well as diseased rabbits was studied, to see if there were changes in the red or white blood cell count or hemoconcentration of the blood of rabbits affected with mucoid enteritis. The internal organs of diseased and normal rabbits viz. liver, lungs, heart, spleen and kidney and digestive tract were sectioned and studied for histopathological changes. Bone marrow smears and bone sections were also examined in normal and diseased rabbits. A questionnaire was prepared and sent to rabbit raisers in Kansas in order to collect additional data and information on the disease.

In all the above transmission studies, the disease could not be produced. Blood studies did not show any apparent change in values except slight hemoconcentration in two diseased rabbits. This may be attributed to loss of fluid from the body because of diarrhea. Bone marrow smears and bone sections did not show any apparent change. Tissue sections revealed no changes except in the cecum and colon which appeared to have a moderate infiltration of leucocytes and sloughing of mucosa and edema of the sub-mucosa.

The presence of a virus was not demonstrated in chick embryos or mice after inoculation of filtrates. Coccidia were not found either in fecal samples or in tissue sections of digestive tract.

Survey studies made available additional information on the incidence, occurrence, susceptibility of different breeds, puberty period in females, incubation period, course, symptoms, mortality, control and treatment of mucoid enteritis.

Streptococcus bovis organism was isolated in five out of eight diseased rabbits which had a property of green-pigment production and was mucous in nature.

It was not possible to duplicate the work of Bargen who was able to reproduce diarrhea in rabbits with streptococci isolated from the intestinal scrapings of humans suffering from chronic ulcerative colitis.

Further studies as to the etiology such as bacteria, virus, rickettsia, fungi or impaired metabolism, therefore must be undertaken before arriving at final conclusion.